Synthetic Biology of Novel Thermophilic Bacteria for Enhanced Production of Ethanol

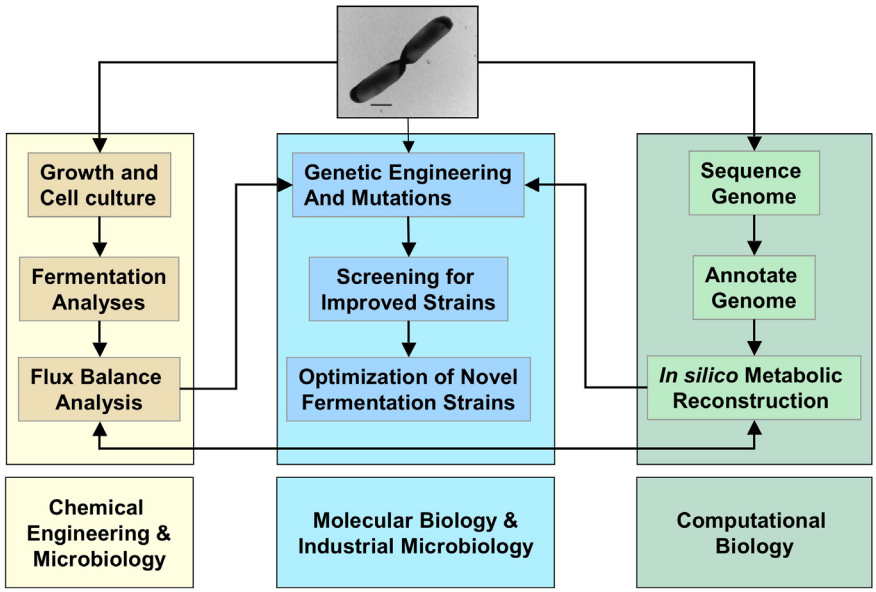

Figure 1: Synthetic Biology is an interdisciplinary approach to design and engineering of biological systems

Sandians are mapping and engineering metabolic pathways for optimum breakdown of cellulose in biomass

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Biomass "energy crops" and agricultural waste are preferred long-term solutions for renewable, cheap, and globally-available lignocellulosic feedstocks for biofuels. However, certain technological challenges must be addressed to make the production of biofuels from biomass more efficient and economical in order to replace fossil fuels. The most promising production process (termed SSF for simultaneous saccharification and fermentation) combines the enzymatic hydrolysis of cellulose recovered from biomass to simple sugars, followed by downstream fermentation of the sugars in a single bioreactor. However, SSF is limited in practice because the optimal process conditions for the individual steps differ. Ideally, the SSF process should be carried out at temperatures > 60°C to improve the rate of fuel production, and to decrease byproduct formation resulting in unwanted contamination. This is incompatible with current technologies.

For example, yeasts commonly used for fermentation produce ethanol from C6 sugars (e.g., glucose) at room temperature, and cannot use C5 sugars (e.g., xylose) which form 30-40% of hemicellulosic plant matter. Moreover, industrial enzymatic hydrolysis of cellulose to simpler sugars employs enzymes that have temperature optima of around 55°C, which is much higher than what yeast can tolerate in the downstream fermentation step. Finally, the microbes that utilize both C5 and C6 sugars, unlike yeast, cannot tolerate high ethanol concentrations (>4% by volume). *Geobacillus thermoglucosidasius* M10EXG (*Gth*), a thermophilic bacterium, overcomes some of these limitations. It has an optimal growth temperature of 60°C, can ferment both C5 and C6 sugars, and tolerates ethanol concentrations of up to 10% (by volume), which makes it an ideal microbe to metabolically engineer for improved ethanol production.

Sandia is, therefore, working on improving ethanol production in *Gth* using synthetic biology- a combination of molecular biology, metabolic engineering, computational analysis and microbiology (Figure 1). A basic understanding of the operational pathways is essential to aid engineering; to accomplish that, researchers use metabolic flux analysis (MFA), a high-throughput technology to quantitatively track metabolic pathway activity and determine overall enzymatic function in cells. ^{13}C-labeled glucose and isotopomer flux balance models were used to determine the fluxes through the central metabolic pathways of *Gth* (Figure 2), thus enabling the determination of the theoretical maximum of ethanol production that could be achieved using pathway engineering. Such engineering can be aided by the availability of the genome of an organism; towards that end, Sandia has sequenced *Gth* using a combination of high-throughput pyrosequencing and traditional

Sanger sequencing. As currently annotated, the genome of *Gth* is 3.8 Mb (Figure 3), and is shown to contain at least 4300 open reading frames comprising protein coding genes and other accessory genes. Metabolic pathways reconstruction is being currently completed for *Gth*. This interdisciplinary approach is not only useful in engineering native pathways, but also for 'designing' a non-native pathway (Figure 4) that could convert a central metabolite to higher energy density fuel like butanol.

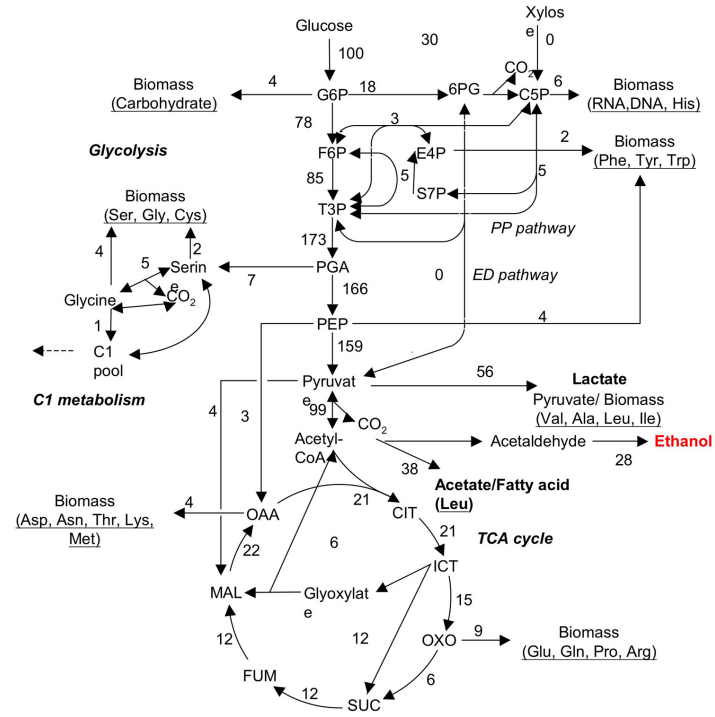

Figure 2: Flux balance analysis of glucose metabolism under micro-aerobic growth of *Gth*.

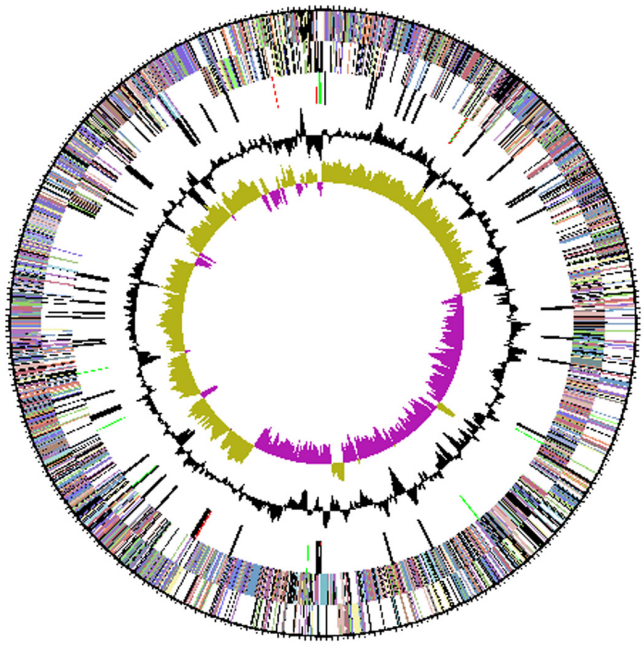

Figure 3: Chromosomal map of *Gth* genes.

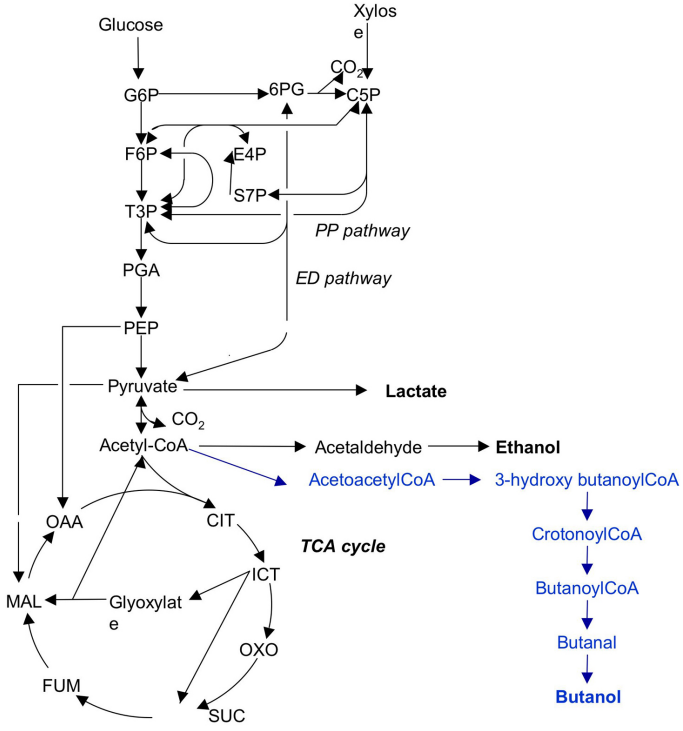

Figure 4: Future direction fuel map: engineering butanol production pathway

Sandia National Laboratories

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